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Disinfection of hatching eggs using low-energy electron beam

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LIST OF ABBREVIATIONS

%	Percent
°C	Degrees Celcius
ABTR	Antibiotic treatment rates
BPW	Buffered peptone water
CFU	Colony forming units
d	Days
DWG	daily weight gain
ebeam	Low-energy electron beam
E. coli	*Escherichia coli*
EFSA	European Food Safety Authority
ECDC	European Centre for Disease Prevention and Control
ECHA	European Chemicals Agency
FWM	First week mortality
g	Grams
h	Hour
keV	Kilo-electronvolt
kg	Kilograms
kGy	Kilogray
log	Logarithmus
m^3	Cubic metre
µm	Micrometre
MALDI-TOF-MS	Matrix-assisted laser-desorption-ionisation – time of flight mass spectrometry
ml	Millilitres
mm	Millimetres
NRGK	Nationales Referenzzentrum für Geflügel- und Kaninchenkrankheiten (National Reference Centre for Poultry and Rabbit Diseases)
OECD	Organisation for Economic Co-operation and Development
OMR	Overall mortality rate
PAA	Peracetic acid
PCA	Plate count agar
PHR	Predicted hatching rate
spp.	Subspecies
THR	True (measured) hatching rate
TVC	Total viable counts
UV	Ultraviolet

1. SUMMARY

1.1. Summary

Microbial contamination of hatching eggs can lead to great losses in poultry production due to rotten eggs, poor hatchability, poor chick quality, reduced growth and decreased performance. Therefore, hatching eggs are sanitised prior to incubation. Hatcheries mostly use chemical disinfectants to treat the hatching eggs. In this study, a new method of hatching egg disinfection using low-energy electron beam (ebeam) was tested under field conditions in a commercial broiler hatchery. The microbial counts on the eggshell surfaces of hatching eggs were compared after treatment with peracetic acid or ebeam. Additionally, broiler production data like hatching rates, first week mortality, medical treatment rates, daily weight gain, overall mortality and carcass condemnation rates at slaughter were compared between the two treatment groups. Treatment of hatching eggs with ebeam led to a significant reduction in microbial counts on the eggshell compared to treatment with peracetic acid. Production data of broilers, hatched from treated eggs, distributed and fattened on different premises, showed no difference between the two treatment groups and thus no negative effect of the ebeam treatment was observed.

Keywords: Hatching egg disinfection, low-energy electron beam (ebeam), peracetic acid, broiler chicken

1.2. Zusammenfassung

Die mikrobielle Kontamination von Bruteiern kann zu grossen Verlusten bei der Geflügelproduktion führen, die auf faulen Eiern, schlechtem Schlupf, schlechter Kükenqualität, vermindertem Wachstum und verminderter Leistung beruhen. Daher werden Bruteier vor der Inkubation desinfiziert. In Brutbetrieben werden die Bruteier meist mit chemischen Desinfektionsmitteln behandelt. In dieser Studie wurde eine neue Methode zur Desinfektion von Bruteiern mit niederenergetischen Elektronenstrahlen (Ebeam) unter Feldbedingungen in einer kommerziellen Brutanlage für Broiler getestet. Die Keimzahlen auf den Eierschalenoberflächen von Bruteiern wurden nach Behandlung mit Peressigsäure oder Ebeam verglichen. Zusätzlich wurden Daten zur Broilererzeugung wie Schlupfraten, Sterblichkeit in der ersten Lebenswoche, medizinische Behandlungsraten, tägliche Gewichtszunahme, Gesamtmortalität und Schlachtkörperausschussrate bei der Schlachtung zwischen den beiden Behandlungsgruppen verglichen. Die Behandlung von Bruteiern mit Ebeam führte zu einer signifikanten Verringerung der Keimzahl auf der Eischale im Vergleich zur Behandlung mit Peressigsäure. Produktionsdaten von Broilern, die aus behandelten Eiern geschlüpft, in verschiedenen Betrieben verteilt und gemästet wurden, zeigten keinen Unterschied zwischen den beiden Behandlungsgruppen, womit kein negativer Effekt der Behandlung mit Ebeam beobachtet wurde.

Schlüsselwörter: Bruteidesinfektion, niederenergetische Elektronenstrahlen (Ebeam), Peressigsäure, Broiler

2. INTRODUCTION

Microbial contamination of hatching eggs may lead to a lower hatching rate and to poor chick quality. Hatching eggs are therefore sanitised prior to incubation in order to ensure hatching of healthy chicks and to minimize economic losses in the hatchery. The following overview gives information on modern poultry production with a focus on the disinfection of hatching eggs. However, only few recent studies on hatching egg disinfection are available. Numerous publications on microbial contamination of eggs date from the second half of the last century. More recent publications focus on describing technical innovation and new methods of hatching egg sanitation.

2.1. Broiler production in Switzerland

2.1.1. Increase of poultry meat production in Switzerland

The per capita consumption of poultry meat in Switzerland is increasing with 9. kg ready-to-cook meat in 2004 to 12.0 kg by 2016 (Proviande, 2017). This is in accordance with consumption rates that are observed in other countries. The reasons for the worldwide increase in poultry meat production are seen in the growing demand for animal-based protein. Poultry is a more affordable product than red meat due to its lower production costs. Its leanness compared to other fattier meats makes it a popular protein source in a healthy diet, and its consumption is not restricted by any of the world religions (Magdelaine et al., 2008). Poultry meat consumption is therefore on the rise in industrialised and developing countries (OECD and Food and Agriculture Organization of the United Nations, 2016). To meet the rising demand for poultry meat in Switzerland, the domestic production has been continuously increasing from 48.8% in 2004 to a level of self-sufficiency of 57.9% in 2017 (Aviforum, 2018a).

The Swiss domestic broiler production is ensured almost exclusively by four poultry companies (Aviforum, 2018b), operating according to a vertical integration system in which the members of the supply chain are united through a common owner (i.e. the poultry companies). The supply chain typically consists of broiler breeder farms, hatcheries, fattening farms and a processing plant. This way, the integrating poultry company can coordinate the individual production stages in the

chain which leads to a higher production efficiency and a significant reduction in production costs. Other characteristics of the contract production are the guaranteed supply and purchase of hatching eggs, broiler chicks and fattened broilers for the producers (Gloor and Aviforum, 2016). Besides that, fattening farms are checked at least once during each production cycle and advise is given to the poultry producers in order to fulfil the hygiene and quality requirements of the vertically integrated poultry production company.

2.1.2. Hatching egg production in Switzerland

Typically, modern broiler breeding programs are organised in a pyramid structure. The purebred lines (great-grandparent animals) are bred by a poultry genetics company for distinctive traits at only a few facilities worldwide. The purebred lines are cross-bred in order to achieve grandparent stock, which in turn are cross-bred again to get the parent stocks (commonly known as broiler breeders). Broiler breeders are either kept and bred by the poultry genetics company or the integrated poultry production firm. The broiler breeder produce hatching eggs resulting in hybrids (known as broilers) with ideal traits for meat production (Martin, 2015).

As the domestic poultry production in Switzerland is rather small, day-old broiler breeder chicks are imported mostly from France, Germany and the Netherlands, and kept as parent stock in Switzerland where they produce hatching eggs for Swiss broiler production. The hatching eggs are taken to the Swiss hatcheries of the poultry company, from where broiler chicks are distributed to individual poultry farmers (growout farms).

2.2. Microbiology and antimicrobial defences of the chicken egg

2.2.1. Bacteria on the surface and in the content of the chicken eggs

Eggs can become contaminated by bacteria via i) the transovarian route where the albumen or the yolk is contaminated before the egg is laid or ii) through trans-shell penetration of bacteria after the egg has been laid (Bruce and Drysdale, 1994). The ovaries and the oviduct of most healthy hens do not act as a contamination source for microorganisms as 90% of newly laid eggs are free from microorganisms (Brooks and Taylor, 1955). However, there are several bacteria species like

Mycoplasma spp., *Salmonella* spp., and *Staphylococcus aureus* that can infect the ovaries or the oviducts and hence be vertically transmitted into the egg in diseased hens (Mayes and Takeballi, 1983). Additionally, infections of the oviduct with e.g. *Mycoplasma synoviae* lead to an altered eggshell structure with thin shells, increased translucency and cracks (Feberwee et al., 2009). This, in turn, can facilitate trans-shell penetration of other microorganisms.

Eggs are laid with a moist surface into nests where they are almost immediately colonised by bacteria from the environment. It has been shown that at the time of laying, an egg can carry between 300 and 500 colony forming units (CFU) of bacteria on its shell (Cadirci, 2009). This number can rise up to 30'000 CFU on the eggshell surface within one hour after lay with visibly dirty eggs carrying even more bacteria (Cadirci, 2009). At the moment of oviposition, the warm egg is exposed to a colder environment in reaction to which the contents of the egg contract, thereby causing a negative pressure which draws bacteria present on the eggshell surface through the pores of the eggshell. The bacterial penetration is also facilitated in the presence of water or other liquids (Bean and MacLaury, 1959; Berrang et al., 1999; Haines and Moran, 1940). According to Bruce and Drysdale (1994), condensation of vaporised water can occur on the eggshell surface when the egg is moved from a cooler area into an area where ambient temperature and relative humidity are favourable. When the hatching eggs at the broiler breeder farm are removed from the cool egg storage room for transportation, water condensation on the eggshell is possible. The "sweating" provides the moisture that is needed for bacterial penetration. If the eggs are then again moved into a cooler environment (e.g. egg storage room at the hatchery), a negative pressure is created, drawing the superficially located microorganisms into the pores, from where some can penetrate the membrane and enter the albumen (Bean and MacLaury, 1959).

2.2.2. Antimicrobial defences of the chicken egg

Although bacteria can penetrate the eggshell with the help of negative pressure and moisture, the avian egg is not completely without defence. It owns certain chemical and physical mechanisms that prevent the microorganisms from invading. The chemical defences include the albumen with its antimicrobial proteins such as ovotransferrin and lysozyme (Board et al., 1994). The physical mechanisms are barriers like i) the cuticle covering the shell, ii) the eggshell itself and iii) the outer and inner membranes. The cuticle is the outermost layer of the chicken egg. The

proteinatious layer is formed during the final 1- 1.5 hour prior to oviposition in the shell gland pouch (Bain et al., 2013; Sparks, 1994). The cuticle is not an evenly distributed layer, as its thickness can vary between 0.5 to 12.8µm over the surface of the whole egg with some eggs having patchy cuticles or even none at all (Board and Halls, 1973; Solomon et al., 1994). The cuticle plays a role in preventing excessive vaporisation of water as well as waterproofing the egg but also functions as a barrier against microbial penetration (Board and Halls, 1973; Williams et al., 1968). Directly after lay, a cuticle-like substance is plugging the pores maintaining the gaseous diffusion through the eggshell but preventing particles like nest debris from occluding the pores which would lead to anoxia for the embryo (Solomon 1991; Sparks, 1994). However, the cuticle is still immature directly after lay and has to dry first. It was shown in an experiment by Sparks and Board (1985) that 100% of the eggs with an immature cuticle (i.e. <30 seconds after oviposition) were penetrated by bacteria whereas in eggs with a mature (dry) cuticle, only 16% were.

Proceeding inwards from the cuticle, the second layer of the egg is the calcified portion of the eggshell. This true egg shell is not only a barrier but also a shield that protects the developing embryo from mechanical stress, and mainly consists of calcium carbonate with a small percentage of organic matrix (Parsons, 1982). It is divided into three distinct layers: firstly, directly beneath the cuticle lies the vertical crystal layer consisting of calcite crystals with a vertical orientation. Secondly, the palisade layer, which is characterised by the presence of vesicles that give the palisade layer a “spongy” appearance. The palisade layer accounts for the largest part of the cross-sectional length of the calcified eggshell and contributes the most to the shell strength (Solomon et al., 1994). Lastly, the innermost part of the calcified portion of the eggshell is the mammillary knob layer. The initial bonding between the outer membrane and the true shell takes place at the cores of the mammillary knobs which have been shown to consist of a protein-mucopolysaccharide complex. Those cores act as nucleation sites where calcium salts precipitate during eggshell formation in the shell gland pouch and where anchoring fibres of the outer membrane attach to (Simkiss, 1968; Taylor, 1970). It is agreed that the toughness of the shell is positively correlated with the thickness of the shell. However, Williams et al. (1968) showed in their work that shell thickness had no significant influence on the number of eggs penetrated by *Salmonella* Typhimurium, a conclusion later supported by De Reu et al. (2006).

The true shell integrity is disturbed by a number of pores, which, according to Tyler (1953) and Simkiss (1968), range from 7000 to 17000 per egg. The pores are funnel-shaped with a diameter of up to 65 µm at the orifice and 23 µm at the inner end (Simkiss, 1968). They ensure the gas exchange between the developing embryo and the environment.

Attached to the inner surface of the true shell is the outer shell membrane. The paired membranes are formed around the yolk and the albumen during egg formation and are only separated at the blunt end of the egg where the air cell is formed (Solomon et al., 1994). The membranes are roughly 70 µm thick with the outer layer accounting for the larger part with a thickness of approximately 50 µm (Simkiss, 1968). Although being much thinner than the outer membrane, the inner membrane has a tighter meshwork and is, according to Lifshitz (1964), a far more important barrier against microbial penetration than the outer membrane.

2.3. Hatching egg disinfection

2.3.1. The importance of hatching egg disinfection

Microbial contamination of eggs is an important topic in the production of hatching eggs and poses a great economic factor in the poultry industry as it can lead to rotten eggs (so called “bangers” that explode in the incubator), poor hatchability, poor chick quality, reduced growth and decreased performance. Furthermore, the penetration of hatching eggs by infectious agents can lead to their dissemination in the hatchery, to the grow-out flock as well as to the final product. This can be a risk for flock health, and moreover if the germs are human pathogens, the poultry products carrying them may subsequently pose a threat to human health. However, losses due to egg rot, low hatchability and poor quality chicks only occur when the eggshell of contaminated hatching eggs is penetrated by pathogenic bacteria, that can overcome the antibacterial defences of the egg content. Although it is of uppermost importance in modern poultry production to keep hygiene standards at a high level, it is not possible to produce hatching eggs that are free of any microorganisms, as the eggs are laid into nest boxes, where pieces of litter and faeces keep getting carried in by the hens. Hatching eggs therefore always carry a certain microbial flora, which, according to Mayes and Takeballi (1983), also

depends on the geographical location, but mostly consists of gram-positive bacteria originating from dust, soil or faeces.

2.3.2. Methods of hatching egg disinfection

In order to ensure adequate hatching hygiene, hatching eggs must be sanitised before incubation. The following section provides an overview of the different disinfection methods used in a commercial hatchery. The appropriate choice for a particular hatchery depends on different factors such as the size of the operation or the premises at the hatchery.

Earlier methods like ethylene oxide, oiling, washing or treating with heat or antibiotics did not always result in satisfactory reduction of egg rot (Bean and MacLaury, 1959). In the face of the emerging antibiotic resistance problems (European Food Safety Authority and European Centre for Disease Prevention and Control, 2018; Projahn et al., 2016), the use of dipping solutions containing antibiotics, as recommended earlier (Barbour et al., 1985; Bean and MacLaury, 1959), is no longer supported.

The method of fumigation with formaldehyde is widely used as a means of hatching egg disinfection in hatcheries as formaldehyde is known to be an excellent antimicrobial agent with no corrosive action on metals. The biocidal action of formaldehyde is based on its effect on proteins and nucleic acids by being able to form intermolecular cross-linkages in proteins (Cadirci, 2009). However, due to its toxicity it can lead to retarded growth or embryonic death, if the fumigation is not carried out properly (Cadirci, 2009; Zeweil et al., 2015). It is also classified as a category 1B carcinogen and therefore poses a health threat to hatchery workers (ECHA European Chemicals Agency, 2018). Formaldehyde can be applied as a liquid by spraying or dipping the eggs, but most hatcheries apply formaldehyde as a gas prior to incubation which is the most effective way of disinfecting the eggs (Cadirci, 2009). Samberg and Meroz (1995) even recommend disinfecting the hatching eggs the first time directly after collection and the second time within 12 hours after setting. To achieve a satisfactory biocidal effect, several factors have to be taken into account: correct concentrations at the corresponding temperature, the size of the disinfection chamber, the relative humidity, the duration of the fumigation and the presence of organic matter such as blood, faeces, soil or feed

residues on the egg. Therefore, special care must be taken to ensure that the eggs are clean and collected from nests on a regular basis.

Besides formaldehyde, there are many other chemicals that are used as a safe alternative in commercial hatcheries like hydrogen peroxide, quaternary ammonium compounds, chlorhexidine-, phenolic-, and binary ammonium-based solutions (Keïta et al., 2016; Stringfellow et al., 2009). Also, plant-derived substances, such as oregano, cumin or plant-derived antimicrobials (e.g. trans-cinnamaldehyde and eugenol) were able to reduce bacterial contamination on eggshells (Upadhyaya et al., 2015; Zeweil et al., 2015).

Because the commercial hatchery in this study uses peracetic acid (PAA) fumigation to sanitise the eggs prior to incubation, this chemical alternative to formaldehyde is discussed below. Peracetic acid is known to be a highly effective biocide that does not lose its efficacy in the presence of organic matter. Its biocidal effect is due to the ability to denaturate proteins, disrupt cell wall permeability and to oxidise sulfhydryl and sulphur bonds in proteins, enzymes and other metabolites of microorganisms. It is considered as a safe disinfectant because it decomposes into acetic acid, water, oxygen and hydrogen peroxide and leaves no residue (Rutala et al., 2008). Peracetic acid, alone or in combination with other disinfectants, can be used as an effective surface disinfectant as Gehan (2009) and Samberg and Meroz (1995) have shown. Wood et al. (2013) evaluated the sporicidal effect of PAA against spores of *Bacillus anthracis* by fogging surfaces where spores or biological indicators had been deposited. They found that the fogging with peracetic acid solutions was a promising decontamination technology to prevent diseases, caused by spore-forming bacteria. Disinfection of hatching eggs with peracetic acid, has less often been described, but was shown to be an efficient method to sanitise hatching eggs prior to incubation (Rodgers et al., 2001).

2.3.3. Low-energy electron beam

The term “low-energy electron beam” (ebeam) designates a continuous electron beam carrying electrons with an energy dosage below 300 keV. The electron beam is generated in a cathode ray tube, similar to a Braun tube, where a tungsten filament under high voltage emits electrons into a vacuum. The electrons are accelerated and focused into a curtain by a magnetic field and leave the tube by passing through a thin titanium foil window at the end of the vacuum tube (Anonymous, 2019a). The

electrons, carrying low amounts of energy, only have an impact on the surface molecules of the object they impinge on. The ebeam technology uses electrons to alter the structure of molecules by either cutting chemical bonds or creating new chemical bonds. Because of its ability to modify molecular bonds, the ebeam technology has been used in various industrial applications e.g. ink curing, development of plastics, improvement of semiconductor performance and sterilisation of packaging and food (Anonymous, 2019b). Since the number of electrons produced as well as the surface depth with a maximum of 450 µm can be defined precisely, ebeam disinfection is also suitable for delicate objects such as seed. The treatment of organic seed with ebeam was examined as an alternative to the commonly used chemical seed dressings. In 137 trials with winter wheat an effectiveness up to 100% against seed-borne pathogens could be demonstrated. The electrons successfully killed the pathogens on the seed surface without penetrating the seed coat due to its low penetration depth of 10 to 200µm (Röder et al., 2009). ebeam has further been used to inactivate *Salmonella* Typhimurium bacteria without destroying the surface antigen properties (Kogut et al., 2012).

A recently published proof-of-concept study used nanosecond electron beam (a pulsed electron beam generated by the nanosecond URT-0.5 accelerator) on shell and hatching eggs (Sokovnin et al., 2018). This method used 2 treatments of higher energy levels (500 keV) and a maximum penetration depth of 1800 µm (measured in air), which could potentially lead to penetration of the shell up to the inner membranes and the air chamber. By contrast, a once applied ebeam treatment uses lower energy below 300 keV and a maximal penetration depth of 450µm without jeopardy to the egg content. To our knowledge, no studies on the disinfection of hatching eggs using low-energy electron beam have been published to date.

2.4. Production data

2.4.1. Hatching rate and chick quality

At the hatchery, the hatching rate is predicted based on the age of the broiler breeders and the experience (predicted hatching rate PHR). Additionally, poultry genetic companies provide their customers with tables showing the expected hatching rate in accordance to the age of the breeder animals. After hatch and before

delivery to the broiler farms, the chicks are quality checked and sorted. A good quality day-old chick is clean, dry, has clear and bright eyes and no deformities. The navel is completely sealed and clean, without the yolk sac protruding from the navel or scab covering it (Meijerhof, 2015). Good quality chicks are also alert and react to their environment (Decuypere et al., 2001). Different factors such as the breeders age, health and genetics and the management in the hatchery (i.e. egg handling, incubation, chick processing and placement) have an influence on the quality of day-old chicks making the production of good quality chicks a complex matter (Meijerhof, 2015; Vieira and Moran, 1999).

2.4.2. First week mortality (FWM)

The first week mortality at the broiler farm is an important figure in the poultry production chain, as it is both a measure for the quality of the day-old chicks as well as for the farm management. The first week of life poses a great challenge for the chick, which, coming from the hatchery where conditions were kept at very constant level, has to adapt to a new environment. Further it has to actively start feeding to take up nutrients, formerly provided by the yolk sac. The transition from embryonic to independent life requires several physiological changes in the newly hatched chick, which is why the first few days of life are so crucial for its further development.

Yassin et al. (2009) described the potential of the chick to survive the first week as directly related to the quality of day-old broilers. As mentioned above, there are many factors that can have an influence on the quality of day-old broiler chicks thus the chances for a chick to survive the first week are, partly, already determined before the chick arrives at the fattening farm. Due to the many physical changes in the first days the chick is particularly susceptible to ascending navel and yolk sac infections which are the most common cause of FWM in broilers (Cortés et al., 2004). The poultry genetics company Aviagen states that the mortality in chicks of the breed “Ross 308” in the first 7 days should be less than 0.7%, assuming that the chicks are of good quality and the management at the broiler farm is done properly (Aviagen, 2018). Field studies on FWM in broiler chicks conducted in the Netherlands over the years 2004, 2005 and 2006 showed an average FWM of 1.5% (Yassin et al., 2009). As the reasons for poor chick quality and the consequences of incorrect incubation already have been described, the diseases leading to early mortality in broiler chicks are summarised below.

The most common diseases that lead to death in chicks during the first week post-hatch are navel and yolk sac infections (Abdul-Aziz and Barnes, 2018). The yolk sac is retracted into the body cavity of the chick through the navel shortly before hatching. However, sometimes the yolk sac has not fully moved into the body, thereby creating an entry portal for bacteria. Apart from incompletely retracted yolk sacs, unhealed navels also serve as potential entry for bacteria. The bacteria that are commonly isolated from infected navels and yolk sacs are *E. coli*, *Pseudomonas aeruginosa*, *Enterococcus*, and *Proteus*. Following an omphalitis or yolk sac infection, septicaemia can occur, leading to pericarditis, enlarged spleen and liver or severe generalised polyserositis, eventually leading to death approximately three to four days post-hatch (Meijerhof, 2015). In animals where the navel and yolk sac seem unchanged, the portal of bacterial entry can be the respiratory or the digestive system (Abdul-Aziz and Barnes, 2018).

2.4.3. Antibiotic treatment rate (ABTR)

Antibiotics have been used as growth promoters in the European Union for over 50 years. The growing problem of antibiotic resistance (European Food Safety Authority and European Centre for Disease Prevention and Control, 2018), however, led to the conclusion that the use of antimicrobials in food animals is a public health issue. In 2006, antimicrobials other than coccidiostats and histomonostats were removed from the Community Register of authorised feed additives (Castanon, 2007). Since January 1st, 1999, antibiotic growth promoters are prohibited in Switzerland and antibiotics are only allowed for therapeutic reasons (Wanner, 1999). Statistics show, that in Switzerland in average one out of ten broiler flocks has to be treated with antibiotics (Aviforum, 2017).

2.4.4. Overall mortality rate (OMR)

The overall or cumulative mortality rate is the mortality rate during the whole fattening period. For reasons like physiological changes in the chick's body and higher susceptibility to infections mortality rates in the first week of life are higher than in the following weeks of growout. It is influenced by many different factors such as genetics, age and health of broiler breeders, quality of day-old chicks, start-phase of chicks at the farm and generally feed, water and management at the growout farm (Aviagen, 2018; Heier et al., 2002; Peebles et al., 1999). Taking all these factors into account, the poultry production company sets the target numbers

for the weekly and cumulative mortality for their contract farms, as those figures can be individual. A study from Brigden and Riddell (1975) conducted in Canada measured an OMR of 3.8%. A longitudinal study conducted in Norway from 1996 to 1999 reported a median cumulative flock-level mortality rate of 2.9% during growout, with the estimated corresponding weekly mortality rates of 1.1% in the first week and 0.3% in the second to fifth week (Heier et al., 2002). A recent study from France on the mortality in organic flocks, where the animals have range access and are slaughtered at an older age than conventionally reared chicken, showed similar figures with 2.8% mean mortality rates (Souillard et al., 2019), corroborating that FWM has the largest share in the overall mortality.

2.4.5. Daily weight gain (DWG)

Due to highly specialised breeding programmes, a broiler was able to reach a live weight of 1'500 g in 30 days in 2005, compared to 1925, where it took a broiler chicken 120 days to reach the same weight (Bessei, 2006). Aviagen states for its Ross 308 broilers a DWG objective of 18 g on day one, rising up to 67 g on day 18 and reaching a climax of 98 g on day 37, when most conventionally reared broilers are slaughtered. After this timepoint, the DWG would decline to 66 g on day 70 (Aviagen, 2016). In order for broilers to grow to their genetic potential, they require particular feed formulations that change during their lives, based on their nutritional needs during a certain stage of fattening (Martin, 2015). This so-called phase feeding programme contains starter feeds, grower feeds and finisher feeds (Aviagen, 2018; Martin, 2015). A study conducted by Havenstein et al. (1994) showed the importance of the feed by feeding two different broiler strains (one early broiler strain and one modern broiler strain) with diets based on a "modern" recipe from 1991 and one from 1957. The modern diet increased the body weight of both strains by 18-26%.

2.4.6. Carcass condemnation at the slaughterhouse

Depending on the production goal (whole carcass or parts), broiler chickens are slaughtered at 30 to 37 days of age in Switzerland. Monitoring of diseased or dead animals upon arrival and of healthy and diseased animals during the process of slaughter is not only necessary to maintain hygiene regulations and to produce safe foods but also to observe the health status of a flock and assessing the methods of catching and loading at the farm, the transportation to the slaughterhouse as well as

the slaughtering process (Ansong-Danquah, 1987; Barger, 2015; Herenda and Jakel, 1994). In Switzerland, meat inspection service is provided by the cantonal authorities according to Swiss legislation (Anonymous, 2016) and thus independent from the production companies. As the production conditions in conventional poultry production are similar all over the world, the rates of condemned carcasses at slaughter are comparable (Jakob et al., 1998). Studies from Canada, France, England, Germany, Switzerland, Iran and the USA showed similar percentages of condemned carcasses within a range of 0.54% to 2.87% (Lupo et al., 2008). Jakob et al. (1998) published a study on the condemnation reasons of slaughtered broilers from two major Swiss poultry producing companies. They found ascites syndrome (43.5%), bacterial infections, mostly caused by *E. coli* (14.2%), and runting (14.1%) to be the most common reasons for carcass condemnation with an overall condemnation rate of 1%. A more recent study by Lupo et al. (2008), conducted in western France registered an overall condemnation rate of 0.87%, and showed emaciation (42.1%) and congestion (22.1%) to be the most probable reason for condemnation, whereas ascites syndrome accounted for 2.6% of all condemned carcasses. In recent years condemnation rates of healthy looking well-fed broilers with cellulitis lesions due to *E. coli* are on the rise (Kumor et al., 1998; Poulsen et al., 2018).

3. MATERIAL AND METHODS

3.1. Hatchery and hatching eggs

Although being affiliated to a vertically integrated poultry production firm, the broiler hatchery in this study obtains its eggs mostly from parent stock farms located abroad (Austria, the Netherlands, Germany, France) that do not belong to the vertically integrated poultry production firm. The reason being that this hatchery specifically produces broiler chicks from imported hatching eggs to supplement the domestic broiler production of the poultry production firm, in order to meet the varying demands on the Swiss poultry meat market throughout the year. The hatching eggs in this field trial originated from nine different Ross 308 broiler breeder flocks, aged between 30 and 53 weeks from Austria and the Netherlands.

The ebeam machine (Comet AG, ebeam Technologies, Flamatt, Switzerland) installed at the hatchery was a custom-made prototype to fit into the daily work-flow of the hatchery. However, due to technical difficulties, the originally planned automatic loading of the conveyor belt could not be implemented. Therefore, the loading of the conveyor belt was conducted manually. Prior to the ebeam treatment of hatching eggs, a dummy egg to which dosimetric film was attached, was irradiated with the ebeam machine. This was done in order to measure the electron beam radiation (Risø B3 radiochromic dosimeter film, Tesa, Hamburg) and the X-ray radiation (Gafchromic XR film, Ashland Inc., Covington, Kentucky), to make sure the radiation load was within limits. The voltage was set at 200 keV, resulting in a dosage of 5-50 kGy on the eggshell surface and a penetration depth of about 120 µm. The conveyor belt ran at a velocity of about 1.5 m/min. The first egg sampling at the hatchery was conducted in May 2018. A delivery of hatching eggs consisted of approximately 90’000 hatching eggs of which on each collection, 30 eggs were collected from each i) untreated eggs, ii) eggs treated with peracetic acid (PAA eggs) and iii) eggs treated with ebeam (ebeam eggs), respectively, resulting in a total of 90 hatching eggs per sampling occasion. The sample of untreated eggs was collected approximately 12 hours after the eggs had arrived at the hatchery and were removed directly from the transport egg flats. Upon arrival, the eggs assigned to the peracetic acid group were transferred from the egg flats onto setter trays by the hatchery workers using a vacuum egg lifter and subsequently brought to the fumigation room where they were stored until fumigation, usually taking place

about 24 h after arrival at the hatchery. The fumigation was performed in a 350 m^3 large room using a 1.4% peracetic acid solution. Sampling of the PAA eggs was conducted between 0 h and 12 h after fumigation. The disinfection of the eggs assigned to the ebeam group usually began 12 h after arrival at the hatchery and took two days. Due to technical limitations of the prototype ebeam machine, the hatching eggs had to be placed manually onto the conveyor belt of the machine and, after irradiation, had to be manually removed and placed onto setter trays. The maximum throughput per hour therefore did not exceed 3500 eggs. The sampling of ebeam eggs was usually conducted directly after treatment.

The eggs of each group were aseptically collected by individually placing each egg into a plastic sachet measuring 100 mm x 125 mm (Minigrip®, Flexico, France). The wrapped eggs were placed onto carton egg flats holding 30 eggs each and then transported to the laboratory of the Department of Poultry and Rabbit Diseases (NRGK), at the University of Zurich, Switzerland. On request of the commercial broiler hatchery, the first sampling was conducted as a trial run from collection to hatching, without subsequent samplings. For this, the eggs were collected as described above, incubated and hatched, to ensure that the ebeam treatment had no adverse effects on the hatching rate. After no negative effects were seen in the ebeam group in this trial run, ten consecutive samplings were planned between June 20 and September 13, 2018. One sampling number was not performed because the eggs for the respective treatment group were of different origins, and above all one group consisted of eggs already treated with UV-irradiation at the breeder farm and therefore criteria for comparison within and between groups were not met. Thus, data from the trial run and 9 additional samplings were analysed. Sampling number four had to be discontinued due to technical problems with the ebeam machine that occurred a few hours into treatment. The 30 eggs required for the microbial examination were collected, the remaining eggs that had not received any disinfection yet, however, had to be fumigated with PAA.

3.2. Shell rinse and serial dilution

Each of the hatching eggs collected at the commercial Swiss hatchery was aseptically transferred from the transport plastic bag into a sterile blender bag (Grade Products, England) filled with 20 ml of buffered peptone water (Buffered

peptone water (BPW) (ISO), Thermo Fisher Scientific, Switzerland). The bag containing the egg was then shaken for one minute and the egg subsequently removed from the bag and discarded. A serial ten-fold dilution of the rinsing solution was performed by adding 1 ml of the rinsing solution to a test tube containing 9 ml of peptone water and so forth. Based on data obtained from pre-experiments, irradiated eggs were not expected to bear high amounts of microorganisms, and thus undiluted rinsate was used for further analysis, whereas the rinsing solution of eggs that had been treated with PAA was diluted once (10^{-1}) and the rinsate of untreated eggs was diluted twice (10^{-1} and 10^{-2}). Before sampling, the sterile stomacher bags and test tubes containing buffered peptone water had both been tempered over night at 37°C, since Kawasaki et al. (2008) described that tempered rinsing solution achieved the highest yield of microorganisms in a shell rinsing procedure. Additionally, the incubation of BPW alone in stomacher bags and test tubes served as a sterility test.

3.3. Total viable counts (TVC)

1 ml of each dilution step of the egg rinsing solutions was surface plated onto plate count agar (PCA; Oxoid, Basel, Switzerland) and subsequently incubated at 30°C according to ISO 4833-1:2013 (Anonymous, 2013). After the incubation, the colony forming units (CFU) were enumerated and converted into microbial counts per egg using the following formula:

$$(CFU * dilution\ factor) * 20$$

Where "CFU" designates the number of colony forming units counted on PCA and "dilution factor" stands for the used dilution factor which was either 10^0 (ebeam and PAA), 10^1 (PAA and control) or 10^2 (control). The resulting figure was multiplied by 20, which is the amount of rinsate in a blender bag.

3.4. Hatching of broiler chicks and distribution to growout farms

From batches of on average 90'000 hatching eggs arriving at the commercial hatchery, 90 eggs were collected for bacteriological testing of the eggshell surface. The remaining eggs were sanitised with either PAA fumigation or ebeam and were subsequently incubated as usual. The broiler chicks that hatched from those eggs were quality-checked and delivered to 53 (including trial run) different Swiss broiler growers belonging to the vertically integrated poultry company. In total, 54 broiler flocks were analysed during the study. Of those, 7 flocks were reared for 30 days, one flock for 31 days, 3 flocks for 32 days, 6 flocks for 33 days, 2 flocks for 34 days, one flock for 35 days, 26 flocks for 36 days and 8 flocks for 37 days until slaughter. Both dust and egg shells from the hatcher baskets and boot socks from the growout farms were found to be negative with the ISO 6579-1:2017 method (Anonymous, 2017) in the routine scheme for the control of *Salmonella* in Switzerland (Anonymous, 2018).

3.5. Post-mortem and bacteriological examination of broiler chicks

Upon arrival of the broiler chicks at the fattening farm, farmers were instructed to collect chicks that perished or were culled due to clinical signs in two batches (group 1: days 1-4 and group 2: days 5-7). Chicks were stored on-farm at -20°C and afterwards sent to the NRGK for post-mortem examination.

To assess the cause of the first week mortality, thawed chicks were assayed in batches and subjected to a standard necropsy procedure. In total, 2606 chicks were examined, 1588 from group 1 and 1018 from group 2. It is a common practise in poultry fattening that diseased chicks and those who lag behind in growth are killed by the farmer by dislocation of the neck. In those cases, the underlying cause for the humane killing was assessed. Thus, first, palpation of the chicks' necks determined whether they had died or had been killed by dislocation of the vertebral column. Thereafter, external appearance of the chicks was checked for signs of cannibalism, deformities, or navel infections. Finally, the abdominal cavity was opened and, based on the gross appearance of the organs, cause of disease or death was determined. Decayed carcasses were designated "autolytic" and not further

processed. In few cases, cause of disease or death could not be established, those chicks were categorised as “no cause of death identifiable”.

Three animals from each of the two age groups of a broiler flock were selected for bacteriological examination of yolk sac and/or liver. Organ material was cultured on Columbia agar with 7% sheep blood and bromthymolblue-lactose agar (Oxoid/Thermo Fisher Scientific). Both agars were incubated aerobically for 24 hours at 37 °C. Bacterial identification was done with the Biotyper® MALDI-TOF-MS system (Bruker Daltonics, Billerica, MA, USA / Software: Compass flexControl Version 3.4; MBT Compass 4.1.80).

3.6. Collection of production data

Additional data was collected with a standardised procedure by the integration company i) at the hatchery (predicted hatching rate PHR and true (actually measured) hatching rate TRH), ii) during the whole fattening period (production data of each flock), and iii) at slaughter (confiscation rates). The collected data included: true hatching rate (TRH), first week mortality (FWM) in broiler chicks, antibiotic treatment rate (ABTR) of flock during production, overall mortality rate (OMR) of broilers during one production cycle, daily weight gain (DWG) of broilers, number of confiscated carcasses at slaughter and the reason for the confiscation. The PRH are based on earlier hatching rates and the age of the breeder flock and is an important tool to assess the hatching performance.

The data were collected from May until mid-November, when the last flocks of the field trial were slaughtered.

3.7. Statistical analysis

The statistical analyses were performed with the statistics programme R (The R Foundation for Statistical Computing, Vienna, Austria). A linear mixed-effects model fit by REML was used for the hatching rates, the mortality rates, in the group of PAA and ebeam treated eggs, respectively. Analysis of variance was performed for the average daily weight gain in both groups.

4. RESULTS

4.1. Hatching egg disinfection

The effect of disinfection on microbial counts on the eggshell surface was compared between peracetic acid fumigation and treatment with ebeam. Both in the trial run as well as in the 9 samplings the comparison showed a similar result with ebeam treated eggs carrying significantly fewer microorganisms on the eggshell surface than eggs that had been treated with PAA (Figure 1). Hatching eggs that had not received any form of antimicrobial treatment served as control. The average colony forming unit count of a control egg ranged between $7.00*10^3$ CFU and $1.82*10^6$ CFU per egg (overall average: $2.28*10^5$ CFU per egg). The average CFU count of a fumigated egg was between 0 CFU and $1.54*10^6$ CFU per egg (overall average: $8.73*10^4$ CFU per egg). In eggs that had been treated with ebeam the colony forming unit counts ranged from 0 to a maximum count of $5.28*10^3$ CFU per egg (overall average: $1.58*10^2$ CFU per egg). The average reduction in microbial counts on the eggshell surface after treatment was found to be log 0.78 in PAA and log 3.98 in EB compared to the untreated control. The three groups differ significantly with regard to the logarithmic transformed CFU numbers per egg with all p-values <0.001.

During the whole field study, one egg each was found to be carrying no microorganisms on the eggshell in the control group as well as in the PAA group. With the exception of sampling number four, in all other samplings of ebeam-treated eggs at least three eggs per sampling were found to be carrying no microorganisms on the shell (Table 1).

4.2. Hatching rate

In total, approximately 1’011’000 hatching eggs were assessed. The effect of treating hatching eggs with low energy electron beam on hatching rates recorded post-hatch at the commercial hatchery was compared to the treatment with peracetic acid. The hatching eggs originated from nine different broiler breeder flocks, aged between 30 and 53 weeks. In general, the hatching rate in the ebeam group (86.5 %) was higher than in the PAA group (83.0%) (Figure 2, Table 2).

Compared to the predicted hatching rates PRH based on the experience in the hatchery, the true hatching rates TRH were found to be higher in 11 out of 14 batches for ebeam treated eggs, and in two out of 18 for the PAA eggs (Table 2). Significantly fewer chicks hatched from eggs that had been treated with PAA compared to eggs that had been irradiated ($p<0.001$).

In some runs of this study, the hatching eggs of the ebeam group came from breeder flocks other than in the PAA group. To have a direct comparison, the runs with the same breeder origin are marked in Table 2 (runs 3, 6, 8 and 9).

PAA treated eggs showed generally a lower THR than eggs that had been treated with ebeam which was on average 1% lower (Table 2).

4.3. First week mortality (FWM)

Cause of disease or death within the first week on the growout farm was assessed by necropsy. A total of 2606 chicks between the age of one and seven days were examined in two groups: age group 1 (1-4d) and age group 2 (5-7d) (Table 3). In age group 1, the most common finding in both treatment groups was yolk sac infection with 80% (PAA) and 75% (ebeam), respectively, followed by gout/dehydration (3% and 4%, respectively). The older chicks in age group 2 mostly died or were culled because of *E. coli* septicemia (33%) and yolk sac infection (23% and 22%, respectively), and problems affecting the skeletal system like rickets/osteomyelitis/arthritis (11% and 17%, respectively).

Looking at the FWM (Table 2), average mortality in chicks from the PAA group was 1.11%, compared to 1.07% in the chicks from the ebeam group. This difference is not significant ($p=0.15$).

4.4. Antibiotic treatment rate (ABTR)

The antibiotic treatment rate was recorded. Noted as the number of days a particular flock was under antibiotic treatment during the respective fattening period. Non-antibiotic treatments like acidification of the drinking water was not recorded. Of all 54 broiler flocks, antibiotic treatment was required in eight with a remarkably higher occurence of four treatments in the final run (run 9). With exclusion of the

final run, only four flocks required antibiotic treatment during the first 9 runs (trial run and runs 1-8). The antibiotics were mostly given over four days, in one case the treatment went over five days (Table 2). The differences between the two treatment groups are statistically not significant ($p>0.05$).

4.5. Daily weight gain (DWG)

Table number 2 shows the average daily weight gain of a chick that was recorded at the fattening farm from day one until slaughter. The average daily weight gain in the PAA group was 52.61g (n=380'865), in the ebeam group the average daily weight gain was 52.13g (n=366'315). There was no significant difference between the treatment groups ($p>0.05$).

4.6. Overall mortality rate (OMR)

The overall mortality rate, i.e. mortality over the whole fattening period, was recorded at the fattening farms from day one until slaughter (assessed broilers n=747'180). The lowest mortality rate was recorded in a PAA flock (1.33%, run 3), the highest in an ebeam flock (7.17%, run 8). The average mortality rate in flocks where eggs had been treated with PAA was 7.56%, compared to 4.14% in flocks where eggs had been ebeam-treated (Table 2). The disinfection method did not have a significant impact on the mortality rates during the fattening period ($p=0.5877$).

4.7. Carcass condemnation rate at slaughter

At slaughter, broiler carcasses are condemned due to unfit quality, with special attention given to the occurrence of cellulitis lesions. A total of 595'293 broiler chicken were assessed in this study. 0.019% of the carcasses were excluded from further processing due to failure of internal processes at the slaughterhouse like insufficient bleeding or tearing of the carcasses. 0.79% of the carcasses were condemned due to quality defects such as ascites, trauma, cellulitis, runt or serositis. The number of confiscated carcasses is statistically not significantly related to the method of hatching egg disinfection ($p=0.3099$) (Table 4).

5. DISCUSSION

The importance of a proper hatching egg disinfection lies in the prevention of the distribution of animal and human pathogens, the reduction of bangers in the incubator due to egg rot and the achievement of good hatching rates with good quality chicks (Berrang et al., 1999; Board et al., 1964; Coufal et al., 2003). The goal of this project was to assess low-energy electron beam (ebeam) as a new and safe method of disinfecting hatching eggs on a large scale in a commercial broiler hatchery. The effectiveness was examined by comparing ebeam with peracetic acid fumigation in terms of microbial count reduction on the surface of hatching eggs. Furthermore, data along the supply chain of the vertical integration system was collected, i.e., the hatching rate, on-farm first week mortality of the broiler chicks, overall mortality of broilers during one production cycle, daily weight gain, treatment rate of the flock during the fattening, and the number of condemned carcasses at slaughter.

5.1. Microbial count on the egg surface

Looking at the reduction of microbial counts on the eggshell surface, significant differences could be observed between both disinfection methods (Fig. 1). The disinfection results of the PAA fumigation do not quite coincide with the findings of other workers who found the disinfection with PAA to be highly effective (Cox et al., 2007; Gehan et al., 2009; Rodgers et al., 2001; Samberg and Meroz, 1995). The microbial counts in this work were on average reduced by log 0.78 after treatment with PAA fumigation compared to untreated eggs. A reason for that discrepancy could be insufficient concentrations of PAA particles in the fumigation chamber at the hatchery. Although the manufacturers recommendation for the concentration of a peracetic acid solution for general disinfection of 1% is exceeded with a 1.4% solution, the fumigation chamber at that hatchery is rather large with 350m^3 which could lead to insufficient distribution of the fume potentially resulting in inadequate disinfection of the eggs. It was also seen before that fumigation of chemical compounds has fewer residual effects on the egg shell than dipping (Zeweil et al., 2015).

To date, there are no published studies that investigate the disinfecting effect of low-energy electron beam on hatching eggs. However, different workers have investigated the microbial decontamination of different foods by low-energy

electron beam, including shell eggs (Lung et al., 2015; Serrano et al., 1997; Wong and Kitts, 2003). Table egg studies, however, mainly focus on the extension of shelf life, the changes in physiochemical properties in the treated foods and consumer acceptance, i.e. on the effect the treatment has on the egg as a food and not as a container of potential life. In this study, a prototype ebeam machine with a minimal dosage of 5 kGy and a penetration depth of 120 µm led to an average reduction in microbial counts on the eggshell surface of log 3.98. This is comparable to the study published by Serrano et al. (1997), who achieved a reduction of microbial counts from 10^6 CFU/ml *Salmonella* to levels of less than 10^2 CFU/ml on the eggshell surface of table eggs using X-rays with a minimal dosage of 0.5 kGy. Although that study had a different approach and goal than the present study, it coincides with our findings of a log 3.98 reduction on the surface of hatching eggs. In both PAA and ebeam treated eggs, recontamination of the eggs after sampling is considered to be unlikely. However, it cannot completely be ruled out in PAA eggs, as those were mostly collected several hours after the disinfection had taken place in the hatchery, while the eggs of the ebeam group were normally hand-collected with gloves directly after disinfection.

5.2. Hatching rate

The current study tested the treatment of low energy electron (ebeam) radiation (200 keV) in a large scale study (circa 1'011'000 hatching eggs) in a commercial broiler hatchery with comparison of predicted and true hatching rates to assess adverse effects on developing embryos. PAA eggs were shown not to meet the expected figures in 16 out of 18 cases whereas in only 3 out of 14 cases the ebeam hatching rates lay below the predicted rates (Table 2). The poor outcome compared to predicted hatching rates was attributed to the exceptionally hot summer in Central Europe 2018 (MeteoSchweiz, 2019). Under ideal conditions, the hatching rates for eggs of 30-week-old broiler breeder Ross 308 hens should yield 83.9%, rising up to 87.6% for eggs of 53-week-old hens (Aviagen, 2016). These desirable numbers, however, are not equal to the lower predicted hatching rates the hatchery in this study calculates. Reasons for that discrepancy between ideal and real figures are various. On parent level, the hatching rate is correlated to the age of broiler breeders, with older flocks having lower hatchability rates (Aviagen, 2016; Tona et al., 2004) and the breeder management. Hatchery related factors that impact

hatchability include storage time and temperature, disinfection of the eggs and incubation. Depending on supply and demand, it is common in commercial hatcheries to store hatching eggs for a certain time prior to setting. Eggs in this study were stored between four and six days prior to setting. In addition, there is the number of days the eggs were stored at the broiler breeder farm, which is, however, unknown. It is known that a storage time of 14 days and more has a detrimental impact on the hatchability and the quality of day-old chicks (Cobb & Vantress, 2008; Mather and Laughlin, 1976), especially if the storage temperature is not suitable. Temperatures have to be adapted according to the planned duration of storage. In general, the longer the hatching eggs are stored, the lower the temperatures should be, thereby staying well below "physiological zero" between 19 and 27°C at which embryonic development is minimal (Brake et al., 1997; Funk and Biellier, 1944). The temperatures in the storage room of the hatchery were not recorded during this study but even during the summer season, temperatures inside the storage room remained cool, with ambient temperatures of approximately 20°C. The eggs were incubated in single-stage incubators, where ideal conditions were kept, and suboptimal temperatures can be ruled out due to electronic monitoring. The same also applies to the hatchers where the hatching process of the chicks is monitored closely.

To sum up, ebeam treatment was shown to be non-hazardous to chicken embryos, documented by good hatching rates (Table 2) and lack of malformations in chicks. ebeam can thus be assumed safe for the developing embryo, as even higher energetic nano electron beams (URT 0.5 accelerator, 500 keV) with a higher penetration depth, could not demonstrate morphological signs of radiation damage and no significantly different hatching rates between treated eggs and controls (Sokovnin et al., 2018). The slightly better hatching rates of ebeam treated eggs compared to PAA treated eggs ($p<0.001$) may be due to the manual handling of ebeam eggs, because the prototype ebeam machine could not be fitted with automatic conveyor belt loading. Thus, eggs showing visible cracks or other defects were discharged.

5.3. First week mortality

After hatching, the chicks are graded into good quality chicks and culls. A good quality day-old chick is clean, dry, has clear and bright eyes and no deformities.

The navel is completely sealed and clean, without the yolk sac protruding from the navel or scab covering it. Good quality chicks are also alert and react to their environment (Funk and Irwin, 1955; Raghavan, 1999; from Decuypere et al., 2001). The quality of day-old chicks is dependent on different factors such as the breeders genetics, age and health, and hatchery management (egg handling, incubation, chick processing and placement) (Meijerhof, 2015; Vieira and Moran, 1999). Looking at the egg related factors, studies have shown a significant correlation between breeder age and fresh egg weight which affects the weight of the newly hatched chick and the subsequent weight gain (Peebles et al., 1999; Tufft and Jensen, 1991). Tufft and Jensen (1991) found that chicks from older hens were heavier posthatch and gained significantly more body weight in their first three weeks of life than chicks from younger hens. Peebles et al. (1999) support those findings only partly, as in their study, the chicks of young hens had the lowest body weight at day 0 of growout, compared to chicks from older hens but later in fattening catched up and being even heavier than the chicks from older hens. The increase in egg weight with the age of the hens comes with a larger yolk at the expenses of albumen, which leads to heavier chicks with more energy reserves at the time of hatching (Vieira and Moran, 1999; Tufft and Jensen, 1991). Chicks that hatch from smaller eggs have fewer energy reserves which makes them more vulnerable in the first few days of their lives, especially when the temperatures in the growout stable is too low. This knowledge can be used in practice to support a good start for the chicks by preheating the growout stable in accordance to the expected chick size. This simple measure can already help reducing the mortality rate in the first few days (Meijerhof, 2015).

The recorded mortality rates in the first seven days of fattening (FWM) in this study were 1.11% for the PAA group and 1.07% for the ebeam group, the difference is not significant, $p=0.15$ (Table 2). Aviagen (2018) sets the FWM rate at 1% under ideal conditions. In a field trial on FWM conducted over the years 2004-2006 (Yassin et al., 2009), however, the average mortality rate of 16365 broilers ranged from 0.0% to 3.3% with an average of 1.5%. The main factor influencing the FWM is the quality of day-old chicks, which in turn is related to the breeder age, breeder management, storage time, storage conditions, and the conditions during incubation and hatching (Mather and Laughlin, 1977; Meijerhof, 2015; Peebles et al., 1999; Vieira and Moran, 1999).

In this study, differences could be seen in the causes of death or cull between the

two age groups assessed (group 1: days 1-4 and group 2: days 5-7). While yolk sac infection was the major finding in 75% and 80% of the chicks of age group 1, respectively, in older chicks aged 5-7d findings were more diverse with *E. coli* septicemia, yolk sac infection and rickets/osteomyelitis/arthritis accounting for 72% and 67% percent of the losses, respectively (Table 3). These figures are in accordance with those of Olsen et al. (2012) who also found omphalites, yolk sac infections and polyserositis to be the most common cause of death in chicks in the first week. While the said study (Olsen et al., 2012) did not consider the exact age of the chicks, this study showed that the incidence of infectious diseases like yolk sac infection or polyserositis decreases with increasing age of the chicks.

According to literature, yolk sac infections are mainly caused by *Escherichia coli* (Cortés et al., 2004; Giovanardi et al., 2005; Kemmett et al., 2014) and *Enterococcus* spp. This coincides with our findings in the microbiological examinations of grossly changed organs in the dead chicks where mostly *E. coli*, and to a minor extent *Enterococcus* spp. and *Proteus* spp. were isolated from yolk sac or liver (data not shown).

5.4. Treatment rate

Antibiotic treatment was administered in 8 out of 54 (14.8%) flocks suffering from high mortalities and lameness (Table 2). This number is slightly higher than the average antibiotic treatment rate in Switzerland which is one in ten flocks (10%) (Aviforum, 2017). However, it was noticeable that in one particular run (run 9, September 2018) considerably more flocks (4 out of 8) than usual required treatment. The reason for this unusual cluster is unclear, but was partly attributed to the above average temperatures in September 2018 (MeteoSchweiz, 2019) Excluding that particular run, the treatment rates for nine runs (7.4%) lie below the Swiss average.

5.5. Daily weight gain

The average daily weight gain was 52.61g in PAA flocks and 52.13g in ebeam flocks, a non-significant difference with $p>0.5$ (Table 2). This is not as much as the performance objectives for Ross 308, published by Aviagen, which sets the average daily weight gain at 60.07g in broilers raised for 35 days (Aviagen, 2016). This

reduced daily weight gain is fully intended by the poultry production company, as they do not want their broilers to grow to their full genetic potential for reasons like stocking density, animal welfare and weight and size at slaughter. It must be taken into account, that due to the different fattening periods of 30- 37 days per flock in this field study, the daily weight gain data are not entirely comparable.

5.6. Overall mortality rate

Looking at the overall mortality rate, it is noticeable that the numbers in the ebeam flocks were lower with 4.14% than those in the PAA flocks with 7.56%, but the difference is not significant with p=0.5877 (Table 2). Reasons for that difference can be various. As already mentioned, the breeder flock has a great influence on the performance of their progeny. As in only four out of ten runs the flocks for both treatment methods were identical, the parent flock could have had a noticeable influence in comparing both mortality rates. Additionally, management on the different farms cannot be expected to be exactly the same, resulting in varying mortalities. The European Union (2007) calculates a maximum cumulative daily mortality rate as 1% + 0.06% multiplied by the slaughter age of the flock in days. The target mortality rate of a flock that is grown for 36 days would be therefore 3.16% in order to increase the stocking density. The poultry company attributed the higher overall mortality to the higher FWM in chicks from imported hatching eggs compared to hatching eggs from Swiss broiler breeders, and to the very high summer/ autumn temperature in 2018 (MeteoSchweiz, 2019).

5.7. Condemnation rate

The overall carcass condemnation rate including process related condemnation reasons in 592’544 carcasses in this study was 0.79% on average. There was no statistically significant difference between the two hatching egg treatments (p=0.31). This value lies beneath the condemnation rate of 1% found in a Swiss study from 1998 (Jakob et al, 1998). In both treatment groups cellulitis accounted for the largest part of condemnations, followed by runt (Table 4). Cellulitis was found to be mainly caused by *E. coli* (Messier et al., 1993) and Jakob et al. (1998) also found bacterial infections to be one of the main reasons leading to carcass condemnation, however, the lesion itself is not described in the publication.

5.8. Production data in general

The study was done in a realistic setting in a commercial broiler hatchery, receiving hatching eggs from abroad and distributing broilers to grow-out farms. Because of the small farms in Switzerland (most keeping between 4000 and 22'000 boilers (Aviforum, 2018a) batches of hatching eggs are small, and it was logistically not feasible to equally disinfect eggs of the same broiler breeder flock with the same method, in order not to disrupt the daily business of the broiler hatchery. Direct comparison of hatching rates was only possible in few flocks (Table 2). This might contribute to the nonsignificant differences in production data between PAA and ebeam treated eggs. Further studies should concentrate on higher numbers of hatching eggs from one parent flock, being fattened on one premise in order to being able to minimize factors attributable to the breeder flock and grow-out farm.

Conclusion

Treatment of hatching eggs with ebeam results in an excellent microbial reduction compared to fumigation with peracetic acid. Due to a low penetration depth, the method is safe, with no aberrant effects on the developing embryos, as demonstrated with comparable hatching rates. Fumigation on the other hand can be error-prone, due to insufficient concentration of the solution itself or of the disinfectant in the fumigation chamber. Furthermore, chemical disinfectants can be hazardous to the health of the hatchery workers.

Additional broiler flock production data from the growout farms, such as first week and overall mortality, daily weight gain, antibiotic treatment rates and carcass condemnation rates could not show negative effects in ebeam treated hatching eggs compared to PAA. However, a good disinfection alone was not enough to achieve noticeable improvements throughout the whole production chain in the current field study, as many different factors such as breeder flock, hatchery management and grow-out farm management play a significant role in the performance of broilers.

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7. TABLES AND FIGURES

Table 1: Minimal, maximal, mean and median CFU on the eggshell surface of hatching eggs per run and treatment. T= trial run, PAA= peracetic acid, ebeam= low-energy electron beam.

run	treatment	minimal CFU	maximal CFU	mean CFU	median CFU
T	control (n=30)	15'200	1'584'000	219'026.67	141'000
	PAA (n=28)	1'500	414'400	31'722.14	9'930
	ebeam (n=30)	0	140	24.66	0
1	control (n=30)	27'000	1'638'000	285'133.30	111'000
	PAA (n=29)	3'050	301'600	32'820.34	23'500
	ebeam (n=29)	0	5'280	192.41	0
2	control (n=30)	0	1'630'000	122'586.20	43'000
	PAA (n=28)	6'400	252'000	45'800.00	3'980
	ebeam (n=30)	0	1'520	84.83	0
3	control (n=29)	10'000	352'000	106'482.80	100'000
	PAA (n=29)	0	166'400	17'868.97	9'800
	ebeam (n=30)	0	560	68.67	0
4	control (n=30)	96'000	716'000	389'466.67	391'000
	PAA (n=28)	3'600	299'200	115'721.43	90'900
	ebeam (n=30)	60	1'460	534.67	320
5	control (n=28)	8'000	356'000	6'400.00	35'000
	PAA (n=28)	2'000	108'600	36'257.14	22'500
	ebeam (n=29)	0	520	70.35	0
6	control (n=29)	48'000	444'000	179'586.21	152'000
	PAA (n=14)	46'000	312'000	128'128.57	104'800
	ebeam (n=28)	0	1'760	196.43	30
7	control (n=30)	36'000	1'312'000	269'266.67	190'000
	PAA (n=29)	60	149'400	9'154.00	1'380
	ebeam (n=30)	0	3'640	210.00	80
8	control (n=30)	52'000	1'816'000	311'333.33	211'000
	PAA (n=29)	4'500	1'536'000	303'817.00	78'000
	ebeam (n=30)	0	2'840	154.67	20
9	control (n=29)	56'000	1'280'000	334'689.70	264'000
	PAA (n=29)	1'600	1'106'000	169'845.00	96'300
	ebeam (n=30)	0	700	49.33	20

Table 2: Production data of broiler chicks from either peracetic acid or ebeam treated hatching eggs from different broiler breeder flocks: measured (true) hatching rate (THR) compared to the predicted hatching rates (PHR) (based on the experience of the hatchery), first week mortality (FWM), overall mortality rate (OMR) and average daily weight gain (DWG) of a chick, and antibiotic treatment rates (ABTR) (shown as the ratio of days a flock was treated per days of the fattening period). The tinted areas mark the runs with eggs originating from the same breeder flock in a particular run. T= trial run, PAA= peracetic acid, ebeam= low-energy electron beam.

run	treatment	breeder flock	THR[2] [%]	PHR [%]	ΔTHR/PHR [%]	FWM[3] [%]	OMR[3] [%]	DWG[3] [g]	ABTR[3] [days / fattening days]
T	PAA	HUB	88.4	86	2.4	1.98	4.10	54.29	4/36
		K24	84.8	85	-0.2				
	ebeam	R48	88.5	86	2.5	0.51	4.83	53.80	0
1	PAA	R48	79.8	86	-6.2	1.91	4.73	53.41	0
		HUB	87.3	86	1.3	1.08	4.04	50.87	0
	ebeam	R47	83.9	83	0.9	0.44	2.32	57.75	0
2	PAA	K24	77.8	83	-5.2	1.17	2.77	50.60	4/36
	ebeam	R47	82.7	82	0.7	0.16	2.92	57.45	0
		R48	89.6	86	3.6	0.07	2.61	54.36	0
3	PAA	2390	84.5	86	-1.5	0.58	2.38	50.24	0
		3718	83.1	87	-3.9	0.34	1.33	54.69	0
	ebeam	2390	85.1	86	-0.9	0.37	2.59	53.85	0
4	PAA	2390	81.1	84	-2.9	1.01	2.64	49.42	0
	ebeam[1]	-	-	-	-	-	-	-	0
5	PAA	K24	75.8	82	-6.2	0.63	5.52	52.22	4/36
	ebeam	R48	88.2	85	3.2	0.56	2.72	52.08	0
		HUB	87.7	86	1.7	0.66	3.63	45.13	0

6	PAA	R48	84.4	86	-1.6	0.89	2.08	51.56	0		
		1590	86.4	87	-0.6	0.51	2.04	48.94	0		
		HUB	85.1	86	-0.9	0.89	2.08	51.56	0		
	ebeam	R48	86.7	86	0.7	0.58	2.96	54.13	0		
		K25	88.2	86	2.2	0.98	5.62	47.96	0		
7	PAA	3718	75.5	78	-2.5	1.47	5.59	50.18	0		
		2390	76.1	83	-6.9	1.22	3.40	52.08	0		
	ebeam	1590	89.2	88	1.2	0.39	2.44	50.53	0		
8	PAA	HUB	80.8	86	-5.2	0.64	5.33	58.09	0		
		R48	85.1	86	-0.9	1.91	4.02	52.94	0		
	ebeam	K25	88.2	86	2.2	1.97	6.33	51.26	4/36		
		R48	85.4	86	-0.6	0.45	7.17	50.32	0		
9	PAA	HUB	82.3	85	-2.7	0.32	5.22	54.74	0		
		R49	83.5	85	-1.5	2.05	5.40	55.49	0		
	ebeam	K25	90.7	85	5.7	4.48	6.54	51.57	4/36	0	
		R49	84.3	85	-0.7	3.42	5.32	49.62	4/30[4]	5/34[4]	4/32[4]	0[4]

[1]The ebeam treatment was discontinued in the fourth run due to a technical problem

[2]The difference between the TRH of ebeam compared to peracetic acid treated hatching eggs is higher in 11 out of batches, with on average 1% higher hatchability in ebeam eggs ($p < 0.01$)

[3]The difference between chicks hatching from ebeam compared to peracetic acid treated eggs was not significant ($p > 0.05$)

[4]The chicks from the ebeam treated eggs were distributed to four different fattening farms, therefore resulting in different ABTR for each flock.

Table 3: Necropsy findings in 2606 broiler chicks with reference to their respective age group and treatment.

	Age group 1 1-4 days n=1588		**Age group 2 5-7 days n=1018**	
	PAA **[%]**	ebeam **[%]**	PAA **[%]**	ebeam **[%]**
yolk sac infection	80	75	23	22
colisepticemia	3	3	33	33
rickets/ ostomyelitis/ arthritis	0	0	11	17
gout/ dehydration	3	4	5	3
failure of circulation	1	1	5	5
intestinal pathology	3	4	5	3
deformation	2	3	3	2
trauma	2	2	1	2
no diagnosis	3	3	6	3

Table 4: Condemnation rates, other than process related reasons, of broiler carcasses at the slaughterhouse originating from PAA and ebeam treated hatching eggs, respectively.

	PAA n= 293'862 [%]	**ebeam n=301'431 [%]**
ascites	0.05	0.06
trauma	0.00	0.00
runt	0.13	0.19
cellulitis	1.00	0.41
serositis	0.00	0.02
total	**1.18**	**0.68**

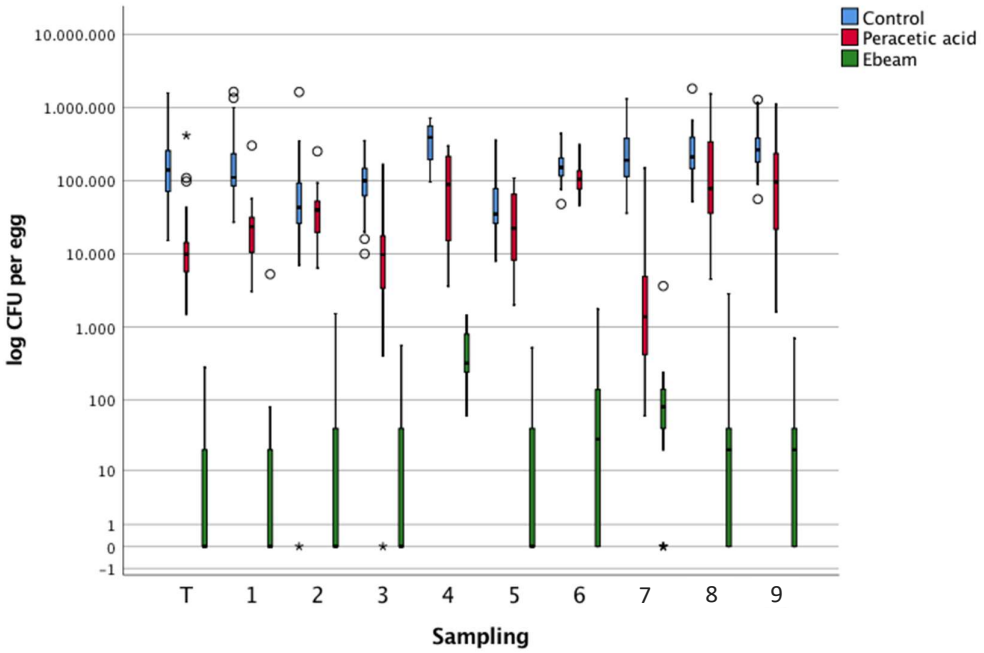

Figure 1: Total viable counts per hatching egg (logarithmic scale) for either untreated (control), or treated with peracetic acid (PAA) or low-energy electron beam (ebeam) eggs (in 10 runs, 30 eggs per treatment were sampled). Numbers of colony forming units (CFU) for control and PAA are arithmetically averaged from two dilution steps. The difference between the three groups is significant ($p<0.001$). T= trial

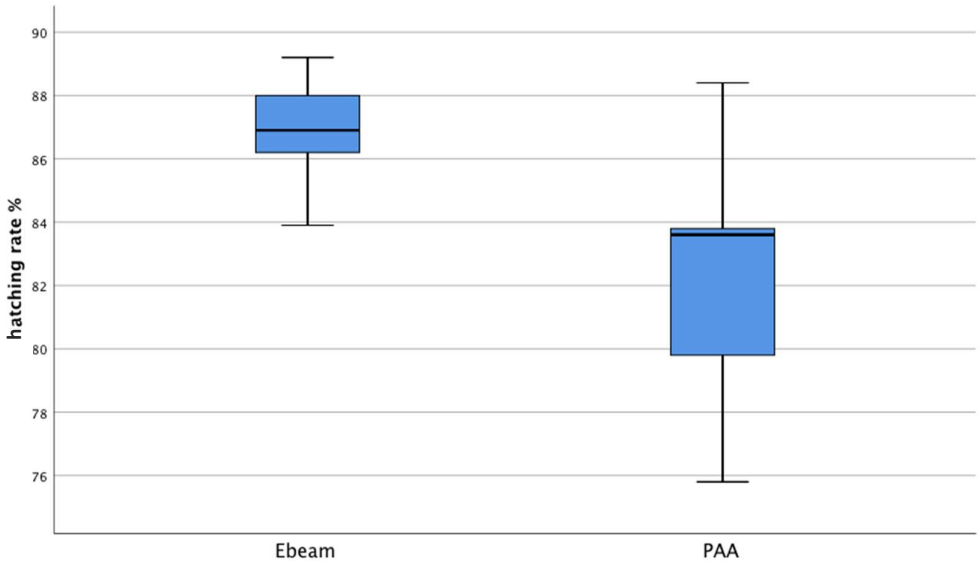

Figure 2: Overall true hatching rates of broiler chicks, hatching from either peracetic acid (PAA) or low-energy electron beam (ebeam) treated eggs of all runs. The difference is significant ($p<0.001$).

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